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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Bruck, et al. August 16, 2001
Serial No.: 09/509,239 Group Art Unit No.: 1648
Filed: March 23, 2000 Examiner: U. Winkler
For: "Fusion Proteins Comprising HIV-1 Tat and/or Nef Proteins"

Assistant Commissioner for Patents
Attn: Chief Draftsman
Washington, D.C. 20231

TRANSMITTAL OF FORMAL DRAWINGS

In response to the Notice of Informal Drawings mailed on July 17, 2000 attached
please find:

- (a) the formal drawings for this application with each sheet indicating the Serial
Number and the Group Art Unit. Number of Sheets: 18
- (b) a copy of the Notice of Informal Drawings.

Respectfully submitted,

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FIG. 1A Map of plasmid pRIT14586

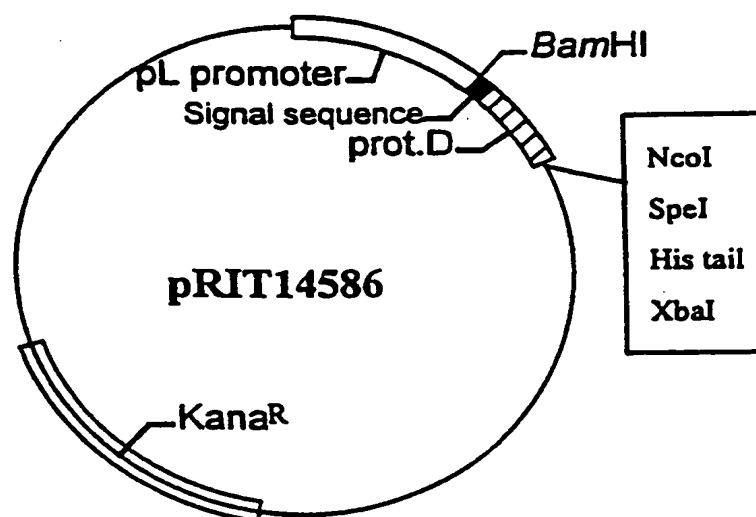


FIG. 1B

Coding sequence of the first 127 amino acids of protein D and multiple cloning site. The signal sequence is underlined.

ATG GAT CCA AAA ACT TTA GCC CTT TCT TTA TTA GCA GCT GGC GTA CTA GCA GGT TGT AGC AGC

Met Asp Pro Lys Thr Leu Ala Leu Ser Leu Leu Ala Ala Gly Val Leu Ala Gly Cys Ser Ser

CAT TCA TCA AAT ATG GCG AAT ACC CAA ATG AAA TCA GAC AAA ATC ATT ATT GCT CAC CGT GGT

His Ser Ser Asn Met Ala Asn Thr Gln Met Lys Ser Asp Lys Ile Ile Ile Ala His Arg Gly

GCT AGC GGT TAT TTA CCA GAG CAT ACG TTA GAA TCT AAA GCA CTT GCT TTT GCA CAA CAG GCT

Ala Ser Gly Tyr Leu Pro Glu His Thr Leu Glu Ser Lys Ala Leu Ala Phe Ala Gln Gln, Ala

GAT TAT TTA GAG CAA GAT TTA GCA ATG ACT AAG GAT GGT CGT TTA GTG GTT ATT CAC GAT CAC

Asp Tyr Leu Glu Gln Asp Leu Ala Met Thr Lys Asp Gly Arg Leu Val Val Ile His Asp His

TTT TTA GAT GGC TTG ACT GAT GTT GCG AAA AAA TTC CCA CAT CGT CAT CGT AAA GAT GGC CGT

Phe Leu Asp Gly Leu Thr Asp Val Ala Lys Lys Phe Pro His Arg His Arg Lys Asp Gly Arg

TAC TAT GTC ATC GAC TTT ACC TTA AAA GAA ATT GAA AGT TTA GAA ATG ACA GAA AAC TTT GAA

Tyr Tyr Val Ile Asp Phe Thr Leu Lys Glu Ile Gln Ser Leu Glu Met Thr Glu Asn Phe Glu

NcoI SpeI XbaI

[illegible]

THE NEW AND THE OLD

The amino acid sequence of Figure 1 relates to Seq. ID no. 7 and the nucleic acid sequence of Figure 1 relates to Seq. ID no. 6.

FIG. 2A

The DNA and amino acid sequences of Nef-His; Tat-His; Nef-Tat-His fusion and mutated Tat is illustrated.

Pichia-expressed constructs (plain constructs)

⇒ Nef - HIS

DNA sequence (Seq. ID. No. 8)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGC
CACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 9)

MGGKWSKSSVVGWPTVRERMRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFVPTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ Tat - HIS

DNA sequence (Seq. ID. No. 10)

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAA
ACTGCTTGTAACCAATTGCTATTGTAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTTC
ATAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
CCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAA

FIG. 2B

TCCCGAGGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCAT
TAA

Protein sequence (Seq. ID. No. 11)

MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRR
PPQGSQTHQVSLSKQPTSQSRGDPTGPKETSGHHHHHH.

⇒ Nef - Tat - HIS

DNA sequence (Seq. ID. No. 12)

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGA
ATGAGACGAGCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAA
AAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGG
CTAGAAGCACAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTA
AGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGG
GGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATC
TACCACACACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTC
AGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAG
GTAGAAGAGGCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCAT
GGAATGGATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCA
TTTCATCACGTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAG
CCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCT
TGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTTCATAACA
AAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCT
CAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGA
GGGGACCCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 13)

^^

MGGKWSKSSVVGWPTVRERMRRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAW
LEAQEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWI
YHTQGYFPDWQNYTPGPGVRYPLTFGWCYKLVPEPDKVEEANKGENTSLLHPVSLH
GMDDPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTA
CTNCYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSR
GDPTGPKETSGHHHHHH.

E.coli-expressed c nstructs (fusion constructs)

⇒ LipoD-Nef-HIS

FIG. 2C

DNA sequence (Seq. ID. No. 14)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
 AGCAGCCATTCATCAAAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
 GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACTGTTAGAATCTAAAGCACTT
 GCTTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
 CGTTTAGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTTGCGAAAAAA
 TTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
 GAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGCAAGTGGTCA
 AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
 GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
 AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
 GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
 GCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAAGGGCTAATT
 CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
 TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTGAGATATCCACTGACCTTT
 GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
 GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
 AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCTAGCATTTTCATCACGTGGCCCGA
 GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCACCATCACCAT
 TAA

Protein sequence of the processed lipidated ProtD-Nef-HIS protein (Seq. ID. No. 15)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMTKD
 GRLVVIHDFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGGKW
 SKSSVVGWPTVRERMRRRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
 EEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQG
 YFPDWQNYTPGPGVRYPLTFGWICYKLVPEPDKVEEANKGENTSLLHPVSLHGMDDP
 EREVLEWRFD SRLAFHHVARELHPEYFKNCTSGHHHHHH.

⇒ LipoD-Nef-Tat-HIS

DNA sequence (Seq. ID. No. 16)

FIG. 2D

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

The Lipidation Signal Sequence is underlined. After processing, the cysteine coded by the TGT codon, indicated with a star, becomes the amino terminal residue which is then modified by covalently bound fatty acids.

*

ATGGATCCAAAACTTTAGCCCTTTCTTTATTAGCAGCTGGCGTACTAGCAGGTTGT
 AGCAGCCATTTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAAATCATTATT
 GCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCTAAAGCACTT
 GCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACTAAGGATGGT
 CGTTTAGTGTTATTTCACGATCACTTTTTAGATGGCTTGACTGATGTTGCGAAAAAA
 TTCCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTTACCTTAAAA
 GAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCATGGGTGGCAAGTGGTCA
 AAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCA
 GCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGAGCAATCACA
 AGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAG
 GAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAG
 GCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATT
 CACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC
 TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCACTGACCTTT
 GGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAA
 GGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGATGACCCTGAG
 AGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCACGTGGCCCGA
 GAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTAGACTA
 GAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAACTGCTTGTAACAATTGCTATTGT
 AAAAAGTGTTGCTTTTCATTGCCAAGTTTGTTCATAACAAAAGCCTTAGGCATCTCC
 TATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCAT
 CAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCG
 AAGGAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence of the processed lipidated ProtD-NEF-TAT-HIS protein (Seq. ID. No. 17)

(Amino-acids corresponding to Prot D fusion partner are in bold)

CSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMTKD
 GRLVVIHDHFLDGLTDVAKKFPHRHRKDGRIYVIDFTLKEIQSLEMTENFETMGGKW
 SKSSVVGWPTVRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEAQE
 EEEVGFVPTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWIYHTQG
 YFPDWQNYTPGPGVRYPLTFGWICYKLVPEPDKVEEANKGENTSLLHPVSLHGMDDP
 EREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTNCY
 CKKCCFHCQVCFITKALGISYGRKKRRQRRRPQGSQTHQVSLSKQPTSQSRGDPTG
 PKETSGHHHHHH.

FIG. 2E

⇒ ProtD-Nef-HIS

DNA sequence (Seq. ID. No. 18)

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACC GTGGTGCTAGCGGTTATTTACCAGAGCATACGTTAGAATCT
AAAGCACTTGC GTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTC GTTTAGTGGTTATTACGATCACTTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACCTTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAAGAAAAGGGGGGACTGGAA
GGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGGCCACCATCAC
CATCACCATTAA

Protein sequence (Seq. ID. No. 19)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIILAHRGASGYLPEHTLESKALAFQAQADYL
EQDLAMTKDGRLLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYYVIDFTLK
EIQSLEMTENFETMGGKWSKSSVVGWPTVRERMRRRAEPAADGVGAASRDL
EKHGAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVPLRPMITYKAAVDLSH
FLKEKGGLEGLIHSQRRQDILDLWIYHTQGYFPDWQNYTPGPGVRYPLTFGW
CYKLVPVEPDKVEEANKGENTSLLHPVSLHGMDDPEREVLEWRFD SRLAFH
HVARELHPEYFKNCTSGHHHHHH.

⇒ ProtD-Nef-Tat-HIS

DNA sequence (Seq. ID. No. 20)

FIG. 2F

Nucleotides corresponding to the Prot D Fusion Partner are in bold.

ATGGATCCAAGCAGCCATTCATCAAATATGGCGAATACCCAAATGAAATCAGACAAA
ATCATTATTGCTCACCGTGGTGCTAGCGGTTATTTACCAGAGCATAACGTTAGAATCT
AAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAAGATTTAGCAATGACT
AAGGATGGTCGTTTAGTGGTTATTCACGATCACTTTTTAGATGGCTTGACTGATGTT
GCGAAAAAATTCCACATCGTCATCGTAAAGATGGCCGTTACTATGTCATCGACTTT
ACCTTAAAGAAATTCAAAGTTTAGAAATGACAGAAAACCTTTGAAACCATGGGTGGC
AAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGA
GCTGAGCCAGCAGCAGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACATGGA
GCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCA
CAAGAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATG
ACTTACAAGGCAGCTGTAGATCTTAGCCACTTTTTTAAAGAAAAGGGGGGACTGGAA
GGGCTAATTCCTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACACA
CAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCA
CTGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAG
GCCAATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGGAT
GACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCAC
GTGGCCCGAGAGCTGCATCCGGAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGAT
CCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACCTGCTTGTACCAAT
TGCTATTGTAAAAGTGTTGCTTTTATTGCCAAGTTTGTTTCATAACAAAAGCCTTA
GGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGT
CAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGGACCCG
ACAGGCCCGAAGGAAACTAGTGGCCACCATCACCATCACCATTAA

Protein sequence (Seq. ID. No. 21)

(Amino-acids corresponding to Prot D fusion partner are in bold)

MDPSSHSSNMANTQMKSDKIIIAHRGASGYLPEHTLESKALAFQAQADYLEQDLAMT
KDGRLLVVIHDHFLDGLTDVAKKFPHRHRKDGRYYVIDFTLKEIQSLEMTENFETMGG
KWSKSSVVGWPTVRRERMRAEPAADGVGAASRDLEKHGAITSSNTAATNAACAWLEA
QEEEEVGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLEGLIHSQRRQDILDWLYHT
QGYFPDWQNYTPGPGVRYPLTFGWICYKLVPEPDKVEEANKGENTSLLHPVSLHGMD
DPEREVLEWRFD SRLAFHHVARELHPEYFKNCTSEPVDPRLEPWKHPGSQPKTACTN
CYCKKCCFHCQVCFITKALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSRGDP
TGPKETSGHHHHHH.

⇒ Tat-MUTANT-HIS

DNA sequence (Seq. ID. No. 22)

FIG. 2G

```

ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATC 40
CAGGAAGTCAGCCTAAACTGCTTGTACCAATTGCTATTG 80
TAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTCATAACA 120
GCTGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGAC 160
AGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGT 200
TTCTCTATCAAAGCAACCCACCTCCCAATCCAAAGGGGAG 240
CCGACAGGCCCGAAGGAACTAGTGGCCACCATCACCATC 280
ACCATTAA 288

```

Protein sequence(Seq. ID. No. 23)

Mutated amino-acids in Tat sequences are in bold.

```

MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFIT 40
AALGISYGRKKRRQRRRPPQGSQTHQVSLSKQPTSQSKGE 80
PTGPKETSGHHHHHH. 95

```

⇒Nef-Tat-Mutant-HIS

DNA sequence(Seq. ID. No. 24)

```

ATGGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGC 40
CTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAGCAGC 80
AGATGGGGTGGGAGCAGCATCTCGAGACCTGGAAAAACAT 120
GGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTG 160
CTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGGTGGG 200
TTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACT 240
TACAAGGCAGCTGTAGATCTTAGCCACTTTTTAAAGAAA 280
AGGGGGGACTGGAAGGGCTAATCACTCCCAACGAAGACA 320
AGATATCCTTGATCTGTGGATCTACCACACACAAGGCTAC 360
TTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGGTCA 400
GATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTACC 440
AGTTGAGCCAGATAAGGTAGAAGAGGCCAATAAAGGAGAG 480
AACACCAGCTTGTTACACCCTGTGAGCCTGCATGGAATGG 520
ATGACCCTGAGAGAGAAGTGTTAGAGTGGAGGTTTGACAG 560
CCGCCTAGCATTTCATCACGTGGCCCGAGAGCTGCATCCG 600
GAGTACTTCAAGAACTGCACTAGTGAGCCAGTAGATCCTA 640
GACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAC 680
TGCTTGTACCAATTGCTATTGTAAAAAGTGTTGCTTTCAT 720
TGCCAAGTTTGTTCATAACAGCTGCCTTAGGCATCTCCT 760
ATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCA 800
AGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC 840
ACCTCCCAATCCAAAGGGGAGCCGACAGGCCCGAAGGAAA 880
CTAGTGGCCACCATCACCATCACCATTAA 909

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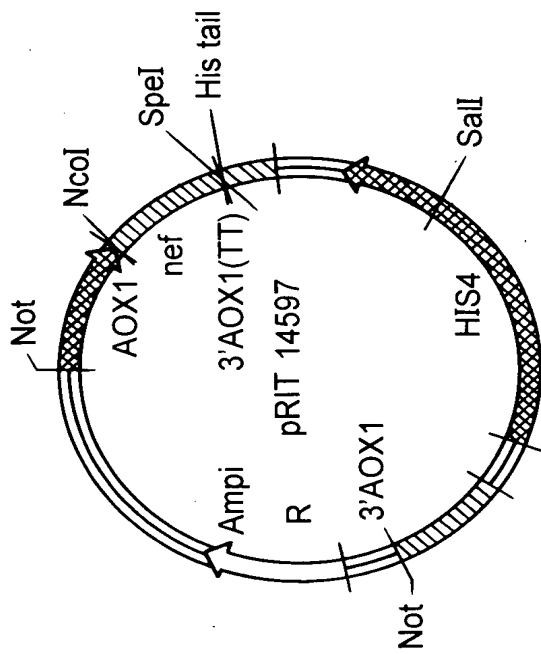
FIG. 2H

Protein sequence (Seq. ID. No. 25)

Mutated amino-acids in Tat sequence are in bold.

MGGKWSKSSVVGWPTVRERMRRAPPAADGVGAASRDLEKH	40
GAITSSNTAATNAACAWLEAQEEEEVGFPVTPQVPLRPMT	80
YKAAVDLSHFLKEKGGLEGLIHSQRRQDILDLWIYHTQGY	120
FPDWQNYTPGPGVRYPLTFGWICYKLVPVEPDKVVEEANKGE	160
NTSLLHPVSLHGMDPEREVLEWRFD SRLAFHHVARELHP	200
EYFKNCTSEPVDPRLEPWKHPSQPKTACTNCYCKKCCFH	240
CQVCFITAALGISYGRKKRRQRRRPPQGSQTHQVSLSKQP	280
TSQSKGEPTGPKETSGHHHHHH.	302

FIG. 3

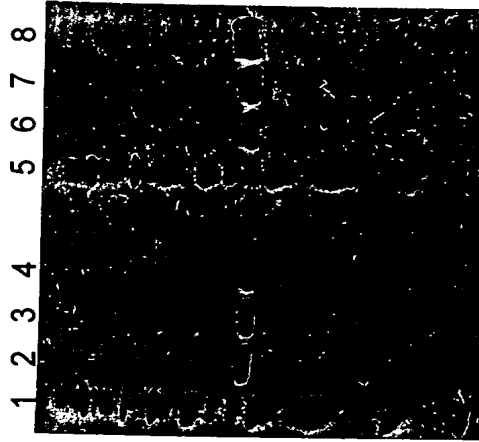


MCS POLYLINKER: *nef* gene inserted between NcoI and SpeI sites.

Acu II *Nco I* *Spe I* *Eco RI*
TTCGAA.ACC.ATGGCCGGGACTAGT.GGC.CAC.CAT.CAC.CAT.CAC.CAT.TAA.CGGAATTC

The amino acid sequence of Figure 3 relates to Seq. ID no. 27 and the nucleic acid sequence of Figure 3 relates to Seq. ID. No.26.

FIG. 4 SDS-PAGE: Nef-Tat-his fusion protein.

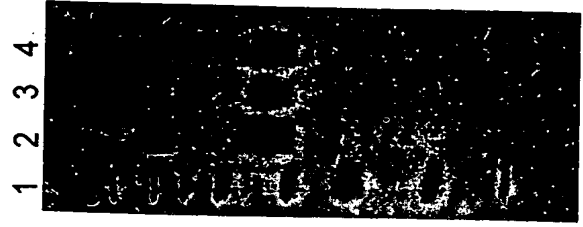


Daiichi Silver Staining



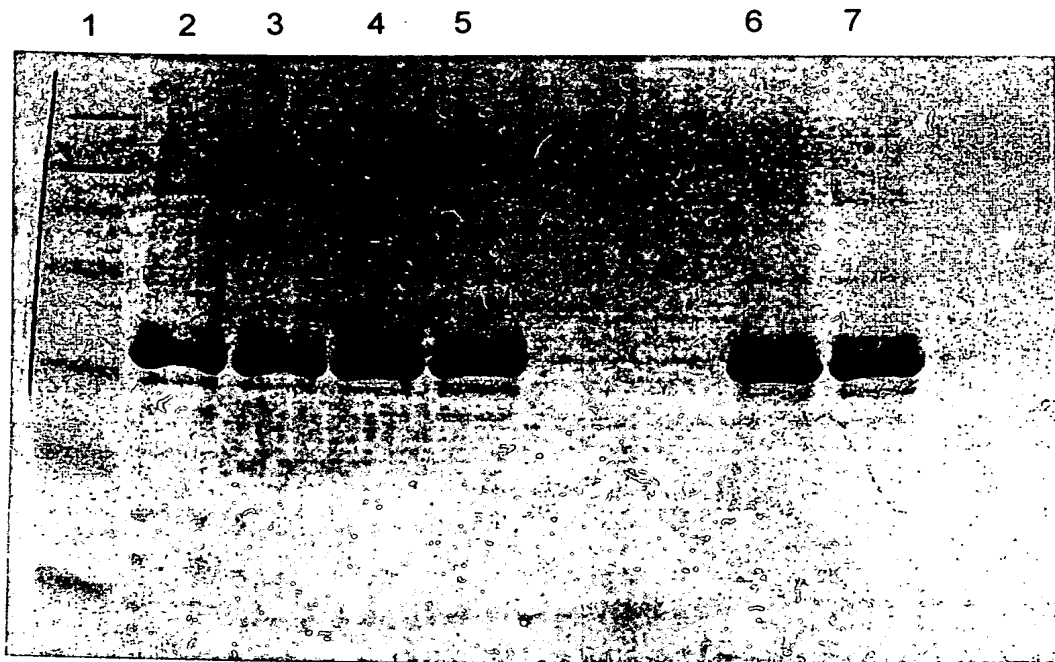
Blot α Nef-Tat (LAS 97340)

- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa
- 2: TNH/23 SP eluate (250 ng)
- 3: TNH/23 Purified bulk (250 ng)
- 4: TNH/22 Purified bulk (250 ng)
- 5: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa
- 6: TNH/23 SP eluate (400 ng)
- 7: TNH/23 Purified bulk (400 ng)
- 8: TNH/22 Purified bulk (400 ng)



Blot Tat2

FIG. 5 SDS-PAGE: Nef-Tat-his fusion protein



Coomassie blue G250

- 1: MW (175/83/62,5/47,5/32,5/25/16,5/6,5 kDa)
- 2: TNH/23 SP eluate (4 μ g)
- 3: TNH/23 Superdex200 eluate (4 μ g)
- 4: TNH/23 Purified bulk (4 μ g)
- 5: TNH/22 Purified bulk (4 μ g)

- 6: TNH/23 Purified bulk (4 μ g) / non reducing conditions
- 7: TNH/22 Purified bulk (4 μ g) / non reducing conditions

FIG. 6A Tat-specific antibody titers and isotypes

group	immunization	midpoint titers					ratio IgG1/IgG2a
		Ig	IgG1	IgG2a	IgG2b		
1	oxydized Tat	353557	135538	98771	98763	1,372	
2	reduced Tat	252275	72087	76273	72014	0,945	
3	oxydized Nef-Tat	246466	179616	60835	53563	2,953	
4	reduced Nef-Tat	91726	73767	30948	20679	2,384	
5	adjuvant only	<4000	<4000	<4000	<4000		

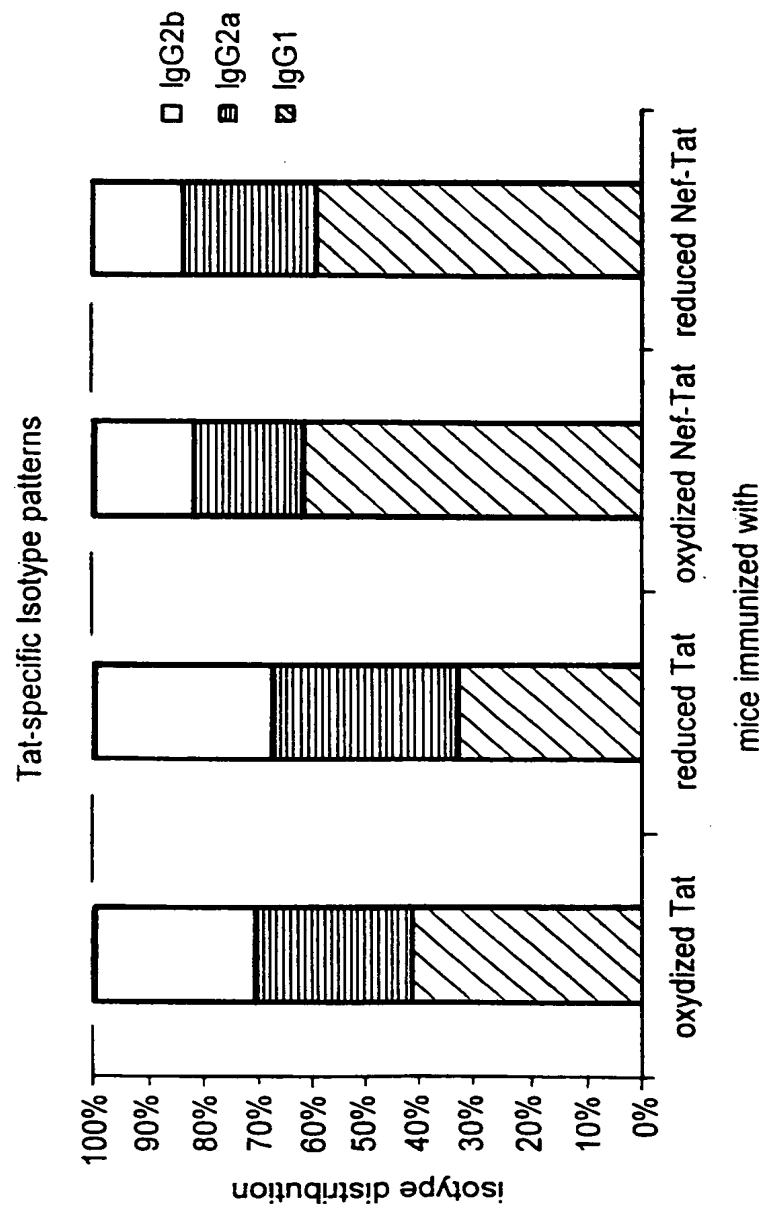


FIG. 6B Tat-specific antibody titers and isotypes

group	immunization	midpoint titers				ratio IgG1/IgG2a
		Ig	IgG1	IgG2a	IgG2b	
1	reduced Tat	212799	123242	62697	55763	1,966
2	reduced Nef-Tat	75676	84046	18449	11692	4,556
3	adjuvant only	<4000	<4000	<4000	<4000	

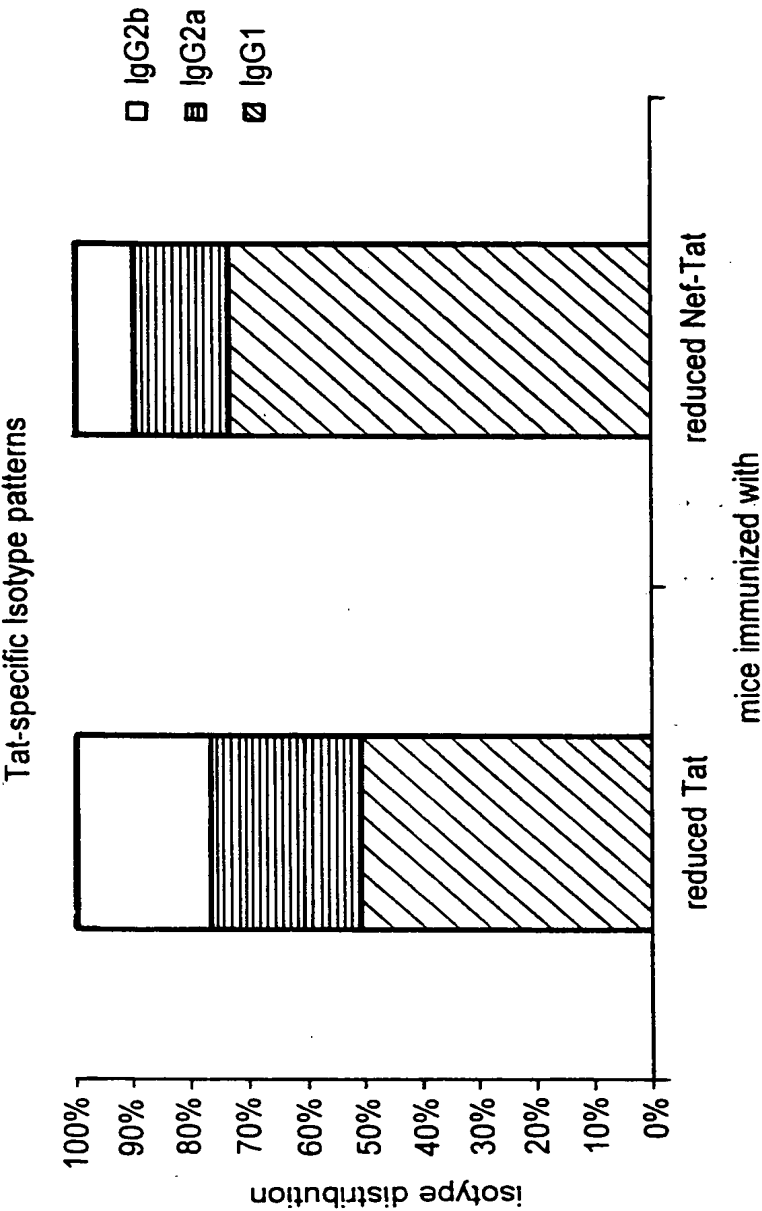


FIG. 7

Antigen-specific lymphoproliferative response of pooled lymph node cells

(3H) Thymidine incorporation in cpm				Data expressed as stimulation index		
	Group 1 reduced Tat	Group 2 reduced Nef-Tat	Group 3 adjuvant only	Group 1 reduced Tat	Group 2 reduced Nef-Tat	Group 3 adjuvant only
reduced Tat 5 μ g/ml	41967	18511	789	140	115	1
1 μ g/ml	37609	32346	415	125	201	1
0.2 μ g/ml	27640	23408	397	92	145	1
reduced Nef-Tat 5 μ g/ml	43882	31694	483	146	197	1
1 μ g/ml	33865	28094	245	113	174	0
0.2 μ g/ml	25079	22891	383	84	142	1
medium	300	161	571	1	1	1

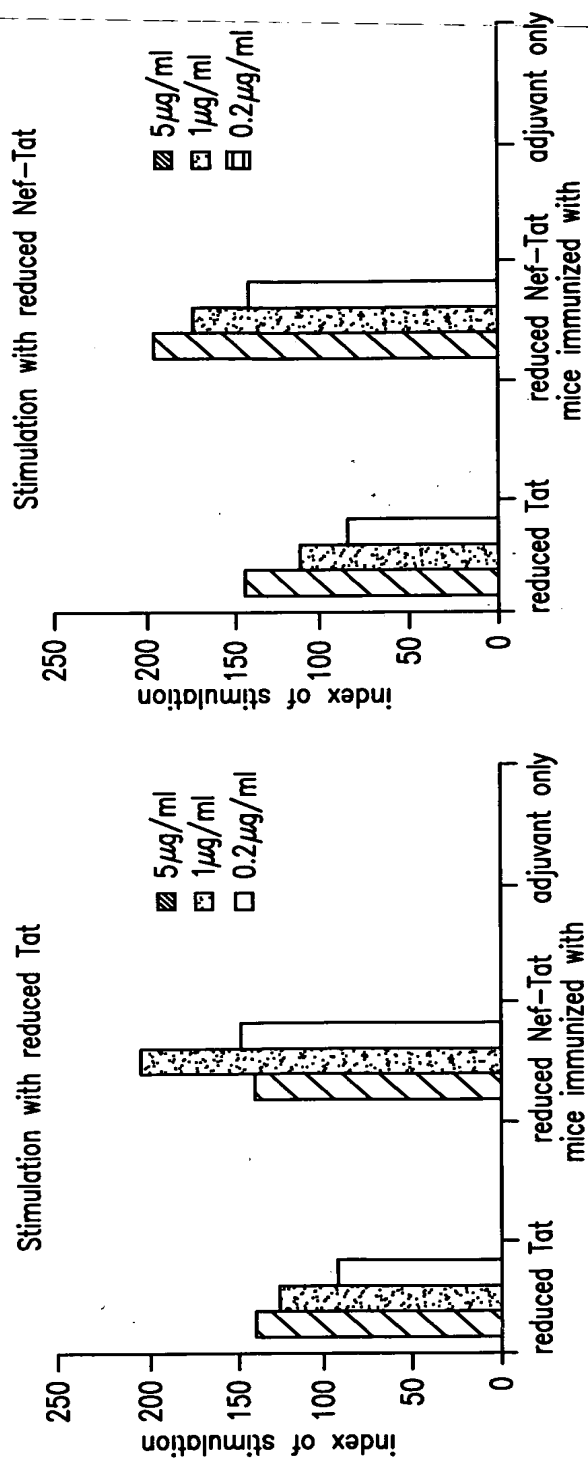


FIG. 8 Cell binding assay

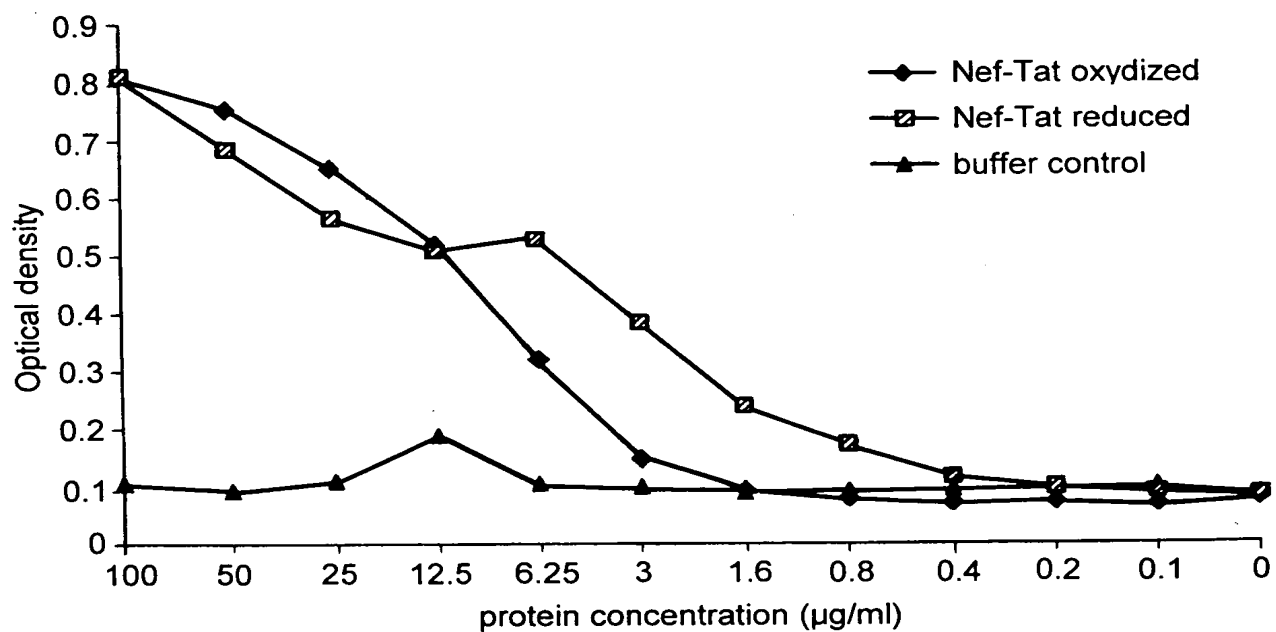
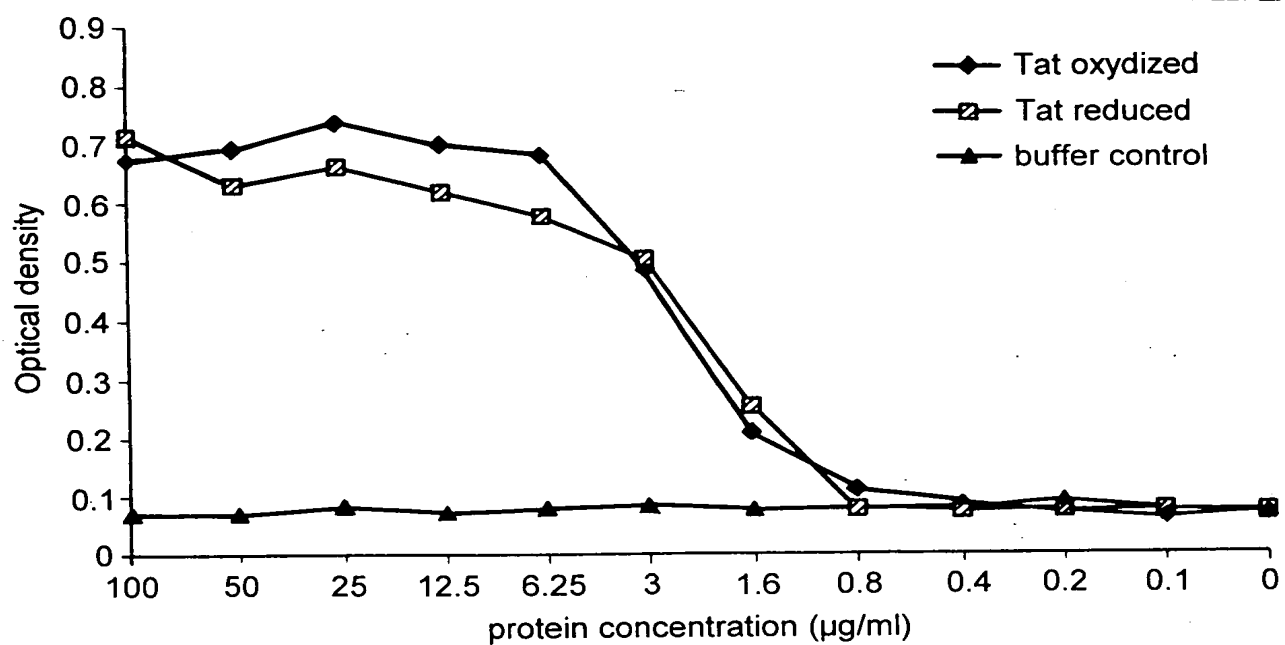


FIG. 9 Inhibition of cell growth

